In re Application of: Yosef YARDEN et al
Serial No.: 10/568,707
Filed: December 14, 2006
Office Action Mailing Date: April 15, 2010

Examiner: PARKIN Jeffrey S.
Group Art Unit: 1648
Attorney Docket: 31570
Confirmation No.: 3267

In the Specification:

Please amend the Paragraph beginning at **Page 20, line 10**, as follows:

Examples of suitable constructs include, but are not limited to pcDNA3, pcDNA3.1 (+/-), pGL3, PzeoSV2 (+/-), pDisplay, pEF/myc/cyto, pCMV/myc/cyto each of which is commercially available from Invitrogen Co. ~~(www.invitrogen.com)~~ (wwwdotinvitrogendotcom). Examples of retroviral vector and packaging systems are those sold by Clontech, San Diego, Calif., including Retro-X vectors pLNCX and pLXSN, which permit cloning into multiple cloning sites and the transgene is transcribed from CMV promoter. Vectors derived from Mo-MuLV are also included such as pBabe, where the transgene is transcribed from the 5'LTR promoter.

Please amend the Paragraph beginning at **Page 21, line 12**, as follows:

Peptide sequences capable of binding Tsg101 or other biomolecular targets of Tal can also be uncovered using computational biology. For example, various peptide sequences derived from Tal can be computationally analyzed for an ability to bind Tsg101 or any other molecular target using a variety of three dimensional computational tools. Software programs useful for displaying three-dimensional structural models, such as RIBBONS (Carson, M., 1997. Methods in Enzymology 277, 25), O (Jones, TA. *et al.*, 1991. Acta Crystallogr. A47, 110), DINO (DINO: Visualizing Structural Biology (2001) ~~http://www.dino3d.org~~wwwdotdino3ddotorg); and QUANTA, INSIGHT, SYBYL, MACROMODE, ICM, MOLMOL, RASMOL and GRASP (reviewed in Kraulis, J., 1991. Appl Crystallogr. 24, 946) can be utilized to model interactions between Tsg101 and prospective peptide sequences to thereby identify peptides which display the highest probability of binding to a specific Tsg101 region. Computational modeling of protein-peptide interactions has been successfully used in rational drug design, for further detail, see Lam et al., 1994. Science 263, 380;

Wlodawer et al., 1993. Ann Rev Biochem. 62, 543; Appelt, 1993. Perspectives in Drug Discovery and Design 1, 23; Erickson, 1993. Perspectives in Drug Discovery and Design 1, 109, -and Mauro MJ. et al., 2002. J Clin Oncol. 20, 325–34.

Please amend the Paragraph beginning at **Page 37, line 25**, as follows:

Synthesis of RNAi molecules suitable for use with the present invention can be effected as follows. First, the Tal mRNA sequence is scanned downstream of the AUG start codon for AA dinucleotide sequences. Occurrence of each AA and the 3' adjacent 19 nucleotides is recorded as potential siRNA target sites. Preferably, siRNA target sites are selected from the open reading frame, as untranslated regions (UTRs) are richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex [Tuschl ChemBiochem. 2:239-245]. It will be appreciated though, that siRNAs directed at untranslated regions may also be effective, as demonstrated for GAPDH wherein siRNA directed at the 5' UTR mediated about 90 % decrease in cellular GAPDH mRNA and completely abolished protein level ~~(www.ambion.com/techlib/tn/91/912.html)~~ (wwwdotambiondotcom/techlib/tn/91/912dothtml).

Please amend the Paragraph beginning at **Page 38, line 4**, as follows:

Second, potential target sites are compared to an appropriate genomic database (e.g., human, mouse, rat etc.) using any sequence alignment software, such as the BLAST software available from the NCBI server ~~(www.ncbi.nlm.nih.gov/BLAST/)~~ (wwwdotncbidotnlmdotnihdotgov/BLAST/). Putative target sites which exhibit significant homology to other coding sequences are filtered out.

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Please amend the Paragraph beginning at **Page 38, line 32**, as follows:

Examples of construction and amplification of synthetic, engineered DNAzymes recognizing single and double-stranded target cleavage sites have been disclosed in U.S. Pat. No. 6,326,174 to Joyce *et al*. DNAzymes of similar design directed against the human Urokinase receptor were recently observed to inhibit Urokinase receptor expression, and successfully inhibit colon cancer cell metastasis in vivo (Itoh *et al* , 20002, Abstract 409, Ann Meeting Am Soc Gen Ther ~~www.asgt.org~~wwwdotasgtdotorg~~)~~. In another application, DNAzymes complementary to bcr-ab1 oncogenes were successful in inhibiting the oncogenes expression in leukemia cells, and lessening relapse rates in autologous bone marrow transplant in cases of CML and ALL.

Please amend the Paragraph beginning at **Page 51, line 13**, as follows:

Yeast-two hybrid assays **-** The full length coding sequence of Tsg101 (GenBank Accession No. NM_006292.) was fused to the LexA DNA binding domain (amino acids 1 to 211) of the pBTM116 bait vector (Constructed from CLONTECH pGBT9 by Fields s and Bartel Proc Natl Acad Sci U S A. 1993 Oct 1;90(19):9186-90). The L40 yeast strain (Invitrogen, Corp. Rhenium Ltd. Israel) was first transformed with pBTM116-Tsg101, tested for auto-activation and then transformed with a human brain cDNA library (CLONTECH, Palo ~~a~~Alto, CA) cloned in pGAD10 vector. All transformations were performed using the lithium acetate method as described in CLONTECH YEAST protocol hand book ~~(http://www.clontech.com/techinfo/manuals/PDF/PT3024-1.pdf)~~ (wwwdotclontechdotcom/techinfo/manuals/PDF/PT3024-1dotpdf). Co-transformants were plated onto Trp-Leu-His selective medium supplemented with 3-aminotriazole (5Mm Sigma St. Louis, MO). His^+ colonies were then assayed for β-galactosidase using a filter lift assay as described in CLONTECH YEAST protocol hand book ~~(http://www.clontech.com/techinfo/manuals/PDF/PT3024-1.pdf)~~

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(wwwdotclontechdotcom/techinfo/manuals/PDF/PT3024-1dotpdf). Positive clones were rescued into bacteria and re-transformed into the L40 yeast strain to confirm interactions. Clones confirmed in this manner were sequenced using the 5' pGAD10 sequence amplifier (CLONTECH, Palo Alto, CA).

Please amend the Paragraph beginning at **Page 56, line 10**, as follows:

The full-length cDNA of human Tal was isolated from a cDNA library derived from a human breast cancer cell line, T47D. As shown in Figure 2a, the open reading frame of human Tal encodes a protein of 723 amino acids. The predicted amino acid sequence of Tal contains an N-terminal leucine-rich repeat (LRR) followed by an ERM domain, coiled-coil region, SAM domain and a C-terminal C3HC4-type RING finger domain (Figure 2b), which is present in many E3 ubiquitin-ligases [Joazeiro and Weissman, (2000) Cell 102, 549-52]. Interestingly, Tal also contains adjacent PTAP and PSAP motifs in a C-terminus portion thereof, and all four amino acids of respective motif located within the late domain of Gag proteins of multiple viruses interacts with the UEV domain of Tsg101 [Pornillos et al., (2002) Nat Struct Biol 9, 812-7; Pornillos et al., (2002) Embo J 21, 2397-406]. Furthermore, mouse Tal (GenBank Accession No. XM149118.3 was identified in the Ensembl data bank, and displayed 86 % nucleic sequence identity, 88.6 % amino acid sequence identity and 91.15 % amino acid sequence homology to the human corresponding sequences as determined by recursive Blast analysis using default parameters (~~www.ncbi.nlm.nih.gov/homloGene~~,wwwdotncbidotnlmdotgov/homloGene) Figure 2c). Similar level of homology was exhibited with rat Tal (GenBank Accession No. XM231157.1, nucleic acid identity 87.9 %, amino acid sequence identity 89.48 % and amino acid sequence homology 92.5 %). As is evident from Figure 2c, Tal is conserved only in vertebrates.